The Effect of Carcinogenic Hydrocarbons and Related Compounds on the Autoxidation of Oils*

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The theory that the carcinogenic hydrocarbons might exert an influence on the tissues by affecting the lipid part of the cell is suggested by the fact that these compounds are insoluble in water but are soluble in most fats and fat solvents. However, very little is known regarding the cellular metabolism of fats, and it is difficult to find a satisfactory system on which to test this theory. One possible mode of action of the carcinogens on fat metabolism might be through their effect on phospholipid oxidation. Rusch and Kline (6) have demonstrated that the oxidation of phospholipids in the presence of glutathione, cysteine, ascorbic acid, thiamin, riboflavin, or pyridoxine can be inhibited by carcinogenic chemicals. It is not known, however, whether the antioxygenic action of the carcinogens is specific for catalyzed phospholipid oxidation or whether other types of fat oxidation are also affected by the hydrocarbons. Accordingly, the following experiments were designed to determine the effect of the carcinogenic chemicals and related compounds on the autoxidation of fats.

Methods

Oils were allowed to oxidize in the presence or absence of various chemicals and the amount of oxidation determined by three methods. Corn oil was used for the first two methods of estimating autoxidation. The various chemicals to be tested were added to the oil at levels of 0.01 and 0.05 per cent and the mixtures exposed to air in Petri dishes at 37° C. for 10 days. At the end of this period the amount of autoxidation was determined. The first method consisted of a modification of the Kreis test as described by Walters, Muers, and Anderson (7). Three cc. of oil diluted with amyl acetate were transferred to a colorimeter tube and 2 cc. of trichloracetic acid solution in amyl acetate (1 gm. per 0.38 cc.) were added and mixed. To this 1 cc. of a 0.5 per cent solution of phloroglucinol in amyl acetate was then added and again thoroughly mixed. A blank was carried through the same steps except that no phloroglucinol was added to the amyl acetate. After standing 30 minutes at room temperature to allow the color to develop, readings were made at 540 mμ with an Evelyn colorimeter. Dilutions of 1 part of corn oil in 100 of amyl acetate were used since this gave a color intensity within the region of maximum accuracy of the colorimeter, and Beer's law was found to hold over the entire range at this dilution. The amount of color depends upon the epihydrin aldehyde formed by oxidation and is proportional to L, or $2 - \log G$, G being the galvanometer reading. As a second method of estimating oxidation, the peroxide numbers were determined by the method of French, Olcott, and Mattill (2).

The Kreis test and peroxide determinations depend upon the formation of products of oxidation and are not a measure of the total oxidation. Therefore, the total oxygen consumption was followed by the manometric method. Two cc. of oil were added to each flask except for ethyl linoleate which was used at 1 cc. levels. With the cod liver oil and lard cod liver oil mixture (1:1), the hydrocarbons and other chemicals were dissolved directly in the oil and 0.005 per cent of copper oleate (8) was added to shorten the induction period of oxidation. No catalyst was used with the corn oil or ethyl linoleate preparations and the carcinogens were dissolved in ether before mixing with these oils. Suction was then applied and the ether removed at 90° C. with continuous vigorous shaking. The presence of naturally occurring antioxidants in corn oil was responsible for a slow rate of oxidation and since the results with this oil were so variable, it was necessary to remove these inhibitors. This was accomplished by placing a flask with corn oil in an oven at 100° C. and bubbling air through for 24 hours. The oil was kept at the same temperature for another 24 hours during which time a stream of nitrogen was passed through it. This treatment resulted in the destruction of most of the inhibitors but was also responsible for other oxidation changes in the oil (5). Gas was liberated for varying periods of time from certain samples of both fresh and heated corn oil. This was prevented in some cases by removing all dissolved gas under a high vacuum. The rate of oxidation was measured for a

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period of 3 to 10 hours with the aid of a Warburg apparatus at 38° C. Except for 9,10-dimethyl-1,2-benzanthracene, which was prepared in our laboratory, the carcinogens and other compounds added to the oils were crystalline materials obtained commercially. The ethyl linoleate was also prepared in our laboratory. Each experiment was repeated 4 to 8 times.

Results

The effect of various compounds on the autoxidation of the corn oil which had been allowed to stand in a warm room at 370° C. for 10 days is given in Table I. It will be noted that while there is some variation in the values of L, the difference is too small to ascribe antioxidant properties to any of the compounds except hydroquinone. Essentially the same results were obtained when the oxidation was checked

Table I: The Effect of Various Compounds on the Autoxidation of Corn Oil

	L values Amounts used		Peroxide No. Amounts used	
Compound	0.01 per cent	0.05 per cent	0.01 per cent	0.05 per cent
Control	0.679	0.659	27.0	27.0
3,4-Benzpyrene	0.733	0.721	31.1	25.1
1,2,5,6-Dibenzanthracene	0.683	0.581	29.9	25.6
Phenanthrene	0.606	0.751	29.7	30.6
Cholesterol	0.688	0.688	29.4	25.9
Desoxycholic acid	0.733	0.683	28.3	28.9
Anthracene	0.673	0.569	30.9	25.6
Dihydroxydiphenyl	0.673	0.648	26.4	25.6
Benzoyl peroxide	0.716	0.756	29.6	36.5
Alloxan	0.694	0.733	23.7	17.4
Hydroquinone	0.553	0.347	16.3	6.9

by the peroxide numbers. It will be noted that alloxan gave a slight inhibiting effect whereas benzoyl peroxide accelerated oxidation somewhat. This action of benzoyl peroxide is similar to that of perbenzoic acid which acts as a pro-oxidant, presumably by destroying the naturally occurring antioxidants present in the oils (4). Other changes were not consistent in all experiments.

In Table II is listed the effect of various substances on the oxygen uptake of cod liver oil. Hydroquinone and alloxan inhibited while benzoyl peroxide accelerated oxidation. Increasing the amount of 9,10-dimethyl-1,2-benzanthracene resulted in a stimulation of oxygen consumption of the lard cod liver oil mixture (Table III). Similar results were obtained with methylcholanthrene on a few runs but such effects were not consistent. With the exception of hydroquinone, most of the chemicals accelerated the oxidation of corn oil (Table IV). This was especially true with alloxan. All samples of oil did not show this stimulating effect, however.

Ethyl linoleate was employed in an effort to minimize the variable results obtained with the crude oils. This ester autoxidizes at a rapid rate and the results of four separate runs are shown in Table V. Freshly prepared ethyl linoleate oxidized at a slower rate than samples which were a few days old. Marked inhibition was always obtained with hydroquinone and, with the exception of one experiment, the same was

Table II: The Effect of Various Compounds on the Autoxidation of Cod Liver Oil *

Compound (0.4 mgm. per flask)	Oxygen consumption (cu. mm.) 1 hr.	2 hr.	3 hr.	4 hr.	5 hr.
Control	80	157	208	278	348
20-Methylcholanthrene	81	144	...	295	...
1,2,5,6-Dibenzanthracene	86	154	...	277	...
3,4-Benzpyrene	81	145	...	294	...
9,10-Dimethyl-1,2-benzanthracene	88	157	...	293	...
Benzanthracene	78	153	210	...	334
Phenanthrene	86	146	210	...	330
Anthracene	91	150	208	...	327
Benzanthraquinone	84	146	220	...	340
Anthraquinone	74	134	210	...	342
Dihydroxydiphenyl	77	148	200	...	316
Desoxycholic acid	74	139	198	...	336
Benzoyl peroxide	84	146	220	...	374
Alloxan	57	105	147	...	235
p-Dimethylaminoazobenzene	68	136	218	...	340
Ascorbic acid	71	130	204	...	310
Hydroquinone	30	60	79	...	123

* 2 cc. cod liver oil per flask—0.005 per cent copper oleate added.

Table III: The Effect of Various Compounds on the Autoxidation of Lard-Cod Liver Oil Mixture (1:1) *

Compound (2 mgm. per flask)	Oxygen consumption (cu. mm.) 1 hr.	2 hr.	3 hr.
Control	130	273	588
20-Methylcholanthrene	132	291	572
1,2,5,6-Dibenzanthracene	126	272	584
3,4-Benzpyrene	127	261	540
9,10-Dimethyl-1,2-benzanthracene	144	308	668
Hydroquinone	25	38	85

* 2 cc. mixture per flask—0.005 per cent copper oleate added.

true for ascorbic acid. In the latter case, however, an initial inhibitory phase of 2 hours was followed by a period of stimulation. The antioxygenic qualities of ascorbic acid on oils have been described by Golumbic and Mattill (3). A mild inhibitory effect was also observed with dibenzanthracene. A marked stimulation was noted with alloxan and a less conspicuous acceleration was observed with aminoazotoluene and desoxycholic acid. In general, the oxidation of freshly prepared ethyl linoleate was accelerated by most hy-

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drocarbons while that of older samples was inhibited (Table V).

TABLE IV: THE EFFECT OF VARIOUS COMPOUNDS ON THE AUTOXIDATION OF CORN OIL *

Compound (0.1 mgm. per flask)	Oxygen consumption (cu. mm.) 1 hr.	2 hr.	3 hr.
Control	65	159	312
3,4-Benzpyrene	104	233	382
20-Methylcholanthrene	102	222	364
1,2,5,6-Dibenzanthracene	47	148	300
9,10-Dimethyl-1,2-benzanthracene	74	197	346
Anthracene	88	194	331
Phenanthrene	88	195	330
Cholesterol	95	210	357
Alloxan	126	255	405
Benzoyl peroxide	98	214	361
p-Dimethylaminoazobenzene	78	175	300
Aminoazotoluene	82	182	308
Hydroquinone	6	14	17

* 2 cc. corn oil per flask.

TABLE V: THE EFFECT OF VARIOUS COMPOUNDS ON THE AUTOXIDATION OF ETHYL LINOLEATE *

Compound	Oxygen consumption 4 hours—cu. mm. Age of oil from time of preparation			
(0.5 mgm. per flask)	1 day	2 days	4 days	20 days
Control	175	410	460	624
3,4-Benzpyrene	208	505	300	324
1,2,5,6-Dibenzanthracene	156	214	312	288
20-Methylcholanthrene	250	505	204	487
9,10-Dimethyl-1,2-benzanthracene	328	515	369	581
Aminoazotoluene	401	661	586	589
Phenanthrene	289	529	384	359
Benzanthracene	306	495	355	572
Desoxycholic acid	462	578	596	494
Benzoyl peroxide	272	497	378	603
Alloxan	2,821	1,920	2,102	...
Hydroquinone	0	12	10	54
Ascorbic acid	20	129	48	631

* 1 cc. ethyl linoleate per flask.

DISCUSSION

These experiments demonstrate that the oxidation of oils and phospholipids differs in at least two respects. Carcinogenic hydrocarbons and related compounds consistently cause a marked inhibition of catalyzed phospholipid oxidation whereas the effect on oil is variable and appears to depend on a variety of factors. These results add emphasis to the inhibitory effect of the carcinogens on the phospholipid-ascorbic acid system (6). Hydroquinone and similar agents are the only substances shown to inhibit both types of reaction. The antioxygenic activity of hydroquinone, for many chemical reactions, is well known and a considerable literature concerning it has resulted (1). Furthermore, ascorbic acid catalyzes the oxidation of phospholipids but curtails the autoxidation of oils (3, 6). This may be the result of differences in solubility, since ascorbic acid is soluble in the aqueous phospholipid substrate and only very slightly soluble in the oils.

The variable results obtained with the hydrocarbons on different samples of oil might be due to several factors. Each sample of oil varies considerably in both the amount of naturally occurring inhibitors and in the degree of previous oxidation. Variations resulting from differences in age of the oil are also observed. When ethyl linoleate is employed, most of the hydrocarbons stimulate oxidation of fresh preparations but inhibit it in older samples. The oxidation of cod liver oil is inhibited by alloxan while that of corn oil and ethyl linoleate is accelerated. The reason for this is not clear.

SUMMARY

The effect of carcinogenic and related compounds on the autoxidation of certain animal and vegetable oils was investigated. The results were variable and depended upon several factors. It is doubtful whether the observed effects have any influence on the mechanism of cancer formation.

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